

Local production of total IgE and specific antibodies to the house dust mite in adenoid tissue

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Adenoids are known as immunosecretory organs and those in atopic children present cellular and cytokine profiles different from those of non-atopic children. We hypothesized that locally produced total IgE and allergen-specific antibodies could be involved in the inflammatory responses in adenoid tissue. Local productions of total IgE and *Der-matophagoides pteronyssinus* (DP)-specific IgE, IgA, IgG1, and IgG4 antibodies were evaluated, as well as their relationships with the markers of allergic inflammation within adenoid tissue. Eighteen atopic subjects, who were sensitized to more than one common aeroallergen, and 22 non-atopic subjects undergoing adenotonsillectomy, were recruited. Immunoassays using adenoid tissue homogenate were performed to quantify the levels of total IgE, eosinophil cationic protein (ECP), and mast cell tryptase. DP-specific IgE, IgA, IgG1, and IgG4 antibodies, soluble IL-2 receptors (sIL-2R), soluble CD23 (sCD23), and IL-6 were measured by ELISA. All parameters measured in adenoid tissue homogenate were presented as a ratio to the albumin level found in the adenoid. Median level of total IgE in adenoid tissue homogenate was significantly higher in atopic individuals than in non-atopic individuals. Median values of DP-specific IgE and IgA antibodies were significantly higher in atopics than in non-atopics ($p = 0.001$, $p = 0.006$, respectively), while no differences were seen in DP-specific IgG1 and IgG4 antibodies. ECP and sCD23 levels in adenoid homogenate were significantly higher in atopics than in non-atopics ($p = 0.026$, $p = 0.048$, respectively), while no significant differences were noted in tryptase, sIL-2R, and IL-6 levels. The levels of DP-specific IgE, IgA, IgG1, and IgG4 antibodies in adenoid homogenate correlated significantly with ECP levels, but not with those of sIL-2R, sCD23, and IL-6. The presence of total IgE and DP-specific antibodies in adenoid tissue was confirmed to be more prominent in atopics. In conclusion, locally-produced total IgE and DP-specific antibodies may contribute to eosinophilic inflammation in adenoid tissue in atopic children.

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Abbreviations: BAL, bronchoalveolar lavage; DP, *Dermatophagoides pteronyssinus*; ECP, eosinophil cationic protein; ELISA, enzyme-linked immunosorbent assay; HDM, house dust mites; MAST-CLA, multiple allergosorbent chemiluminescence assay; sCD23, soluble CD23; sIL-2R, soluble IL-2 receptor.

Atopy refers to the tendency to develop an exaggerated IgE response, i.e., a predisposition to develop allergic diseases including rhinitis, asthma, eczema, and food allergies (1). However, it is not clear which factors control the possible

manifestation and severity of allergic diseases. Among the factors that appear to control the manifestation of the disease, the allergen sensitization route (2) and the regulation and production of IgE locally within the target organ (1) may be considered.

Adenoids are located at the entrance of the respiratory tract. Adenoids and nasal mucosa represent the first site of contact with air-borne antigens before they are inhaled into the respiratory tract. They belong to the lymphoepithelial secretory immune system and constitute part of the Waldeyer's ring, or nasopharynx-associated lymphoid tissue, which provides B cells to mucosal effector sites. It is well known that these lymphoid organs contain IgA-expressing B cells, which migrate to the upper airway mucosa, lacrimal glands, and salivary glands (3). Recently, Papatziamos et al. demonstrated the presence of IgE-bound plasma cells in the adenoids of atopic children. This suggests that adenoids are involved in IgE-mediated sensitization, with local differentiation of IgE-producing plasma cells constituting a probable source of mucosal B cells for the upper airways (4).

Difference in cell population between atopic and non-atopic children is another indication of a potential role for the adenoid during allergic sensitization in the airways. A few studies have shown that adenoids from atopic children present cellular and cytokine profiles different from that of non-atopic children and that eosinophils could be involved in adenoid inflammation (5, 6). However, to the best of our knowledge, there has been little attempt to detect and quantify the production of specific IgE to allergen in adenoid tissue and its correlation with inflammatory markers, specifically presenting the allergic inflammation.

The purpose of this study was to detect locally-produced total IgE and allergen-specific antibodies and their involvement in the inflammatory responses in adenoid tissue in atopic and non-atopic children. The subjects were divided into two groups according to atopy status, to include a sensitized state to the house dust mite, *Dermaphagoides pteronyssinus*. To evaluate the role of total IgE and *Dermaphagoides pteronyssinus* (DP)-specific antibodies on allergic inflammation in the adenoid tissue, total IgE, DP-specific IgE, IgA, IgG1, and IgG4 levels were measured using ELISA. Key inflammatory markers such as ECP, tryptase, sIL-2R (soluble IL-2 receptor), sCD23 (soluble CD23), and IL-6 were also measured and the relationship between DP-specific antibodies was evaluated.

Materials and methods

Subjects

Forty children participated in this study; 26 boys and 14 girls with a mean age of 7.9 yr (range 2.8–15). All subjects underwent adenotonsillectomy between June 2007 and July 2007 at the ENT Department of the Kyunghee University Hospital in Seoul, Korea. Participation was by parental informed consent and the study was approved by the local ethics committee.

All patients were referred to the ENT department for their need of operation because of recurrent infections, such as pharyngotonsillitis, rhinosinusitis, adenitis, and otitis media or problems owing to adenotonsillar hypertrophy, such as upper airway obstruction, sleep disorder, abnormal dentofacial growth, and dysphagia. The parents or foster parents of the children were asked to complete an extensive questionnaire. Data were collected on symptoms and signs of the children's disease(s), medication, and family history with respect to allergy, rhinoconjunctivitis, asthma, eczema, smoking habits, and contact with pets. Children with congenital anomalies in the head and neck, Down syndrome, systemic diseases, or those suspected of having congenital or acquired immune deficiency were excluded from this study.

Atopy status was defined as presenting a sufficiently high total IgE for their age (> 10 IU per year of age) and a score of more than 2+ from the MAST-CLA. Patients were excluded if medications containing antihistamines were used intraoperatively or within the preceding week, or if immunosuppressive agents, including steroids, were used intraoperatively or within the preceding 6 wk.

Before surgery, all children underwent a routine ENT examination. Blood samples obtained from patients during the operation were used for the MAST-CLA, to determine serum total IgE concentration using a BN II assay (Dade Berhing, Marburg, Germany), and to count eosinophils using the ADVIA120 Hematology system (Bayer Diagnostics, NY, USA). For MAST-CLA, antigens attached to threads were sequentially incubated with patient serum, enzyme-absorbed anti-IgE, and a photoresponse reagent. The chemical fluorescence generated by each was developed on Polaroid film and scored according to a 5-point system, from 0 to 4. All reactions of grade 2 or higher were scored as positive.

Tissue collection

The adenoids of patients were removed using a curette. The adenoids were thawed and dispersed

in LIPA buffer (Sigma, St. Louis, MO) containing a protease inhibitor (Roche, Indianapolis). They were harvested by centrifugation and the supernatants were kept and stored at -70°C before use. All parameters measured in the adenoid tissue homogenate were presented as a ratio to the albumin level in adenoid, as described previously, in order to correct the dilution factor between individual tissues (7). Albumin levels in adenoid tissue homogenate were measured using a QuantiChrom™ BCP Albumin Assay Kit (BioAssay Systems, Hayward, USA).

Measurement of total IgE and DP-specific IgE, IgA, IgG1, and IgG4 in adenoid tissue homogenate

The level of total IgE was measured using the ImmunoCAP system (Pharmacia), with the confidence range of 0.35–100 kIU_A/L. The level of specific IgE in adenoid tissue was measured by ELISA as described previously (8). In brief, microtiter plates (Immunon II; Dynatech Laboratories, Inc., Chatilly, VA, USA) were coated with DP (2 µg/well, Allergopharma, Germany) and kept overnight at 4°C . After a washing step, the plates were blocked with phosphate buffered saline (PBS) with 3% bovine serum albumin and 0.05% Tween-20. The plates were incubated with a fixed sample dilution (1:5 dilution in blocking buffer) of adenoid tissue homogenate. After washing, the plates were incubated with biotinylated affinity-purified goat anti-IgE antibody (Vector Laboratories, Inc., Burlingame, CA, USA) at 1:1000 dilution (1 µg/mL) for 1 h at room temperature. The plates were washed and then incubated with streptavidin-peroxidase (0.5 µg/mL) for 30 min at room temperature. After washing, *o*-phenylene diamine was added as a substrate solute and the absorbance was measured using a microplate reader. The parameter of DP-specific IgE was presented as the optical density (OD).

For the measurement of DP-specific IgA, IgG1, and IgG4, the procedure was similar to the above method except that biotinylated affinity-purified goat anti-IgA, IgG1, and IgG4 antibody (Sigma, St. Louis, MO, USA) were used and at different sample dilutions (1:40, no dilution, and 1:1000, respectively).

Inflammatory marker assays and albumin level in adenoid tissue homogenate

ECP (diluted 1:5) and tryptase (diluted 1:20) in adenoid homogenates were quantified by fluoro-

immunoassay using the ImmunoCAP system (Pharmacia, Uppsala, Sweden). The minimal detection limits for ECP and tryptase were 2 and 4 ng/mL, respectively. ECP and tryptase concentrations were corrected for albumin content and expressed as nanograms per milligram of albumin. Levels of sIL-2R (Bender MedSystems, Vienna, Austria), IL-6 (R&D System Inc., Minneapolis, MN, USA), and sCD23 (Bender MedSystems, Vienna, Austria) were quantified by enzyme immunoassays.

Statistical analyses

Inflammatory markers, including ECP, tryptase, sIL-2R, sCD23, IL-6, and levels of DP-specific IgE, IgA, IgG1, and IgG4 antibodies, were compared between atopics and non-atopics using Mann–Whitney *U*-tests with SPSS version 12.0 (Chicago, IL, USA). Pearson's correlations were applied to evaluate the relationships among levels of DP-specific antibodies and inflammatory markers. *p*-values of 0.05 or below were regarded as significant.

Results

Clinical characteristics of study subjects

Eighteen patients (45%), composed of 13 boys and 5 girls, were found to have atopy, meaning they had a sufficiently high total IgE for their age (> 10 IU/year of age), and a score of more than 2+ in the MAST-CLA. Sixteen out of the 18 patients had rhinitis and two of them had both rhinitis and asthma. Twenty-two patients (55%) were non-atopic and served as control group.

The characteristics of the study subjects are shown in Table 1. No significant differences were found in age and sex distribution between the two groups ($p > 0.05$, respectively). Atopic patients were most commonly reactive to house dust mites (HDM, 88.9%) and 28% of these atopic patients were reactive to animal (dog and cat) dander.

Total IgE in sera and total eosinophil count levels on peripheral blood of atopics were significantly higher than those of non-atopics ($p < 0.01$, $p = 0.021$, respectively).

Comparison of total IgE and DP-specific IgE, IgA, IgG1, and IgG4 antibodies between atopics and non-atopics

Total IgE levels in sera and adenoid tissue homogenates of the two groups are shown in Fig. 1. Total IgE levels of sera and tissues homogenates were significantly higher in atopics

Table 1. Clinical data of the study subjects

	Atopics (n = 18)	Non-atopics (n = 22)	p-value
Gender (M/F)	13/5	13/9	NS
Age (years)*	8.2 ± 3.5	7.7 ± 3.5	NS
Sensitized allergens on MAST-CLA (positive/negative)	18/0	0/22	<0.01
House dust mite	15/3	0/22	<0.01
Dog hair	4/14	0/22	NS
Cat hair	3/15	0/22	NS
Cormorbid diseases (positive/negative)			
Chronic rhinosinusitis	7/11	9/13	NS
Otitis media effusion	0/18	2/20	NS
Serum total IgE (IU/mL)†	359.4 (37.7–1220)	53.7 (18.4–188)	<0.01
Total eosinophil count (per mm ³) on peripheral blood†	280.6 (20–480)	172.4 (20–490)	0.021

MAST-CLA, multiple allergosorbent chemiluminescence assay; NS, not significant. Statistical comparisons between atopic and non-atopic values were performed using the Mann–Whitney *U*-test.

*Values are expressed as mean ± SD.

†Total IgE and eosinophil count levels are expressed as median values (range).

than in non-atopics ($p < 0.01$, $p < 0.01$, respectively). DP-specific IgE, IgA, IgG1, and IgG4 antibodies of atopics and non-atopics are shown in Fig. 2. The levels of DP-specific IgE and IgA antibodies were significantly higher in atopics than in non-atopics ($p = 0.001$, $p = 0.006$, respectively), while no significant differences were found in DP-specific IgG1 and IgG4 levels ($p = 0.111$, $p = 0.177$, respectively).

Comparison of inflammatory markers between atopics and non-atopics

The levels of ECP, tryptase, sIL-2R, sCD23, and IL-6 in adenoid tissue homogenates between atopics and non-atopics are shown in Fig. 3.

The ECP and sCD23 levels in adenoid tissue were significantly higher in atopics than in non-atopics ($p = 0.026$, $p = 0.048$, respectively), while no significant differences were noted in tryptase, sIL-2R, and IL-6 between the two groups ($p = 0.422$, $p = 0.878$, $p = 0.491$, respectively).

Relationship between inflammatory markers and DP-specific antibody levels in adenoid tissues

The levels of DP-specific IgE, IgA, IgG1, and IgG4 antibodies significantly correlated with the levels of ECP ($r = 0.546$, 0.457 , 0.598 , 0.501 , respectively) (Fig. 4). In addition, the levels of DP-specific IgE, IgA, and IgG antibodies significantly correlated with the levels of tryptase ($r = 0.369$, 0.522 , 0.604 , respectively) in adenoid tissue homogenate, while poor correlations were found with those of IgG1 ($r = 0.355$).

Discussion

This paper is the first investigation to confirm the presence of locally-produced total IgE and DP-specific antibodies within adenoid tissue and showed that DP-specific IgE and IgA levels were strongly associated with atopy status. In addition, levels of DP-specific antibodies significantly correlated with inflammation markers, ECP and tryptase. These findings give further evidence to support the hypothesis of local productions of total IgE and allergen-specific antibodies, as well as ongoing allergic inflammation in the lymphatic tissue of adenoids from atopic children.

As a part of Waldeyer's lymphatic ring, adenoids are probably the site of antigen-induced priming of B cells and the generation site of antigen-specific lymphoid memory cells and

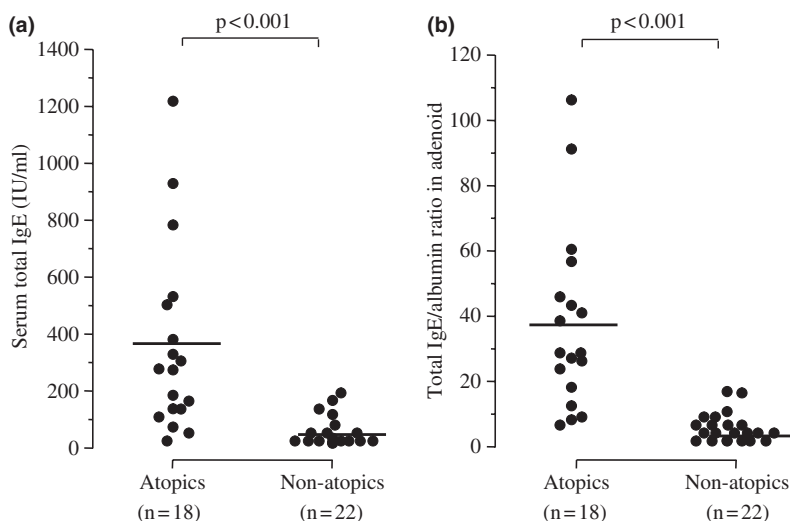


Fig. 1. The levels of total IgE in sera (a) and adenoid tissue homogenates (b) of atopic (n = 18) and non-atopic (n = 22) children. Total IgE in adenoid tissue homogenates were expressed as the ratio to albumin. Group comparisons were performed by Mann–Whitney *U*-test and horizontal bars indicate median values.

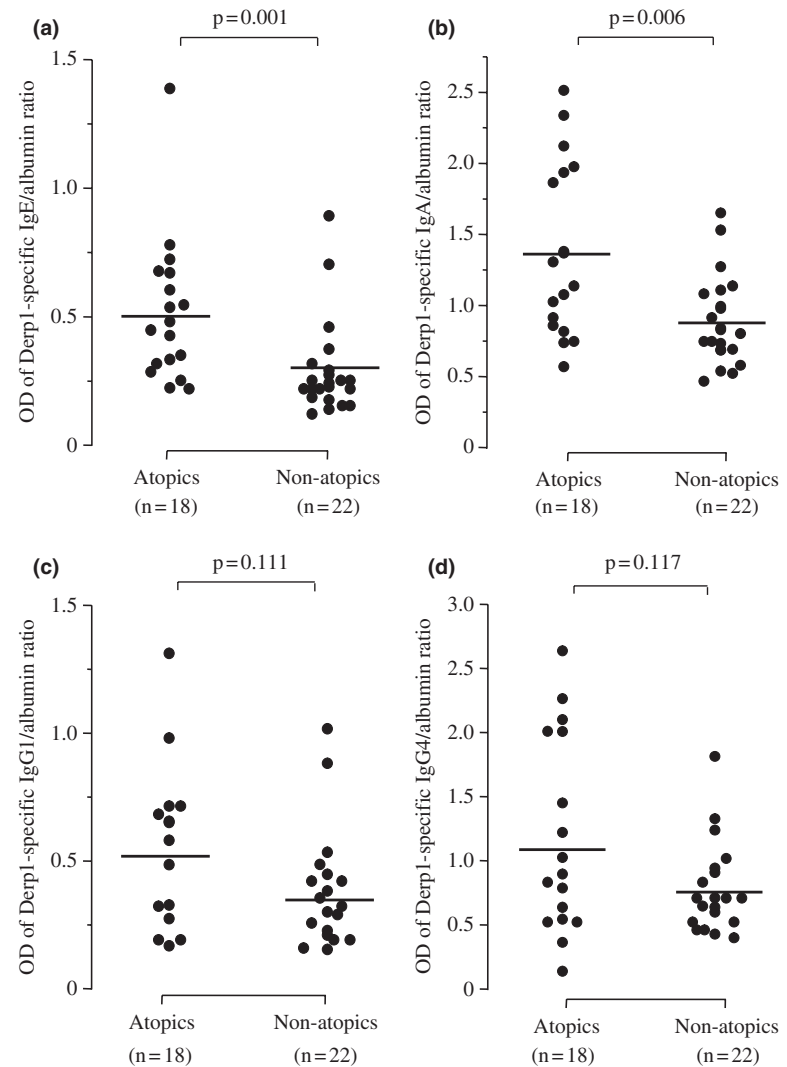


Fig. 2. The levels of DP-specific IgE (a), IgA (b), IgG1 (c), and IgG4 (d) expressed as the ratio to albumin in adenoid tissue homogenates of atopic (n = 18) and non-atopic (n = 22) subjects. Group comparisons were performed using the Mann-Whitney *U*-test and horizontal bars indicate median values.

effector B cells, which thereafter migrate to the upper respiratory tract (9, 10). The local source of IgE-producing cells in the respiratory tract has been debated and remains largely unknown. Recent findings support the concept of local IgE synthesis in the nasal mucosa of allergic rhinitis patients (11). Kleinjan et al. detected IgE-bound B and plasma cells in the mucosa of patients with seasonal and perennial allergic rhinitis (12), and IgE synthesis was demonstrated in cultured nasal mucosa explants from patients with seasonal rhinitis (13). The situation in the adenoid tissue with regard to IgE-bound cells is less characterized. In contrast to the study of Papatziamos et al. (4) and ours, Fokkens et al. could not demonstrate any significant differences regarding IgE-positive cells between adenoids from allergic children and controls (5). The discrepancy could be explained by the difference in study subjects and method applied. Fokkens et al. enrolled study subjects showing high

specific IgE only to food-allergens as well as those sensitized to inhalant allergens, while Papatziamos et al. and this study enrolled children sensitized to inhalant-allergens alone, because adenoid is situated at the entrance of respiratory tract, and it might represent the first contact site for inhalant-allergens. In this study, we confirmed the presence of total IgE and DP-specific antibodies in both adenoid tissue homogenates and sera from atopic and non-atopic children. Atopic children were found to have higher levels of specific antibodies.

Specific IgE, IgA, IgG1, and IgG4 antibodies to the house dust mite in adenoid tissue homogenates between atopic and non-atopic children were compared. Many studies have detected allergen-specific antibodies in sera, nasal polyp tissue, or sputa of patients with allergic diseases and compared their levels with controls (8, 14–17). Aydogan et al. demonstrated that levels of DP-specific IgE, IgG1, and IgG4 in serum were

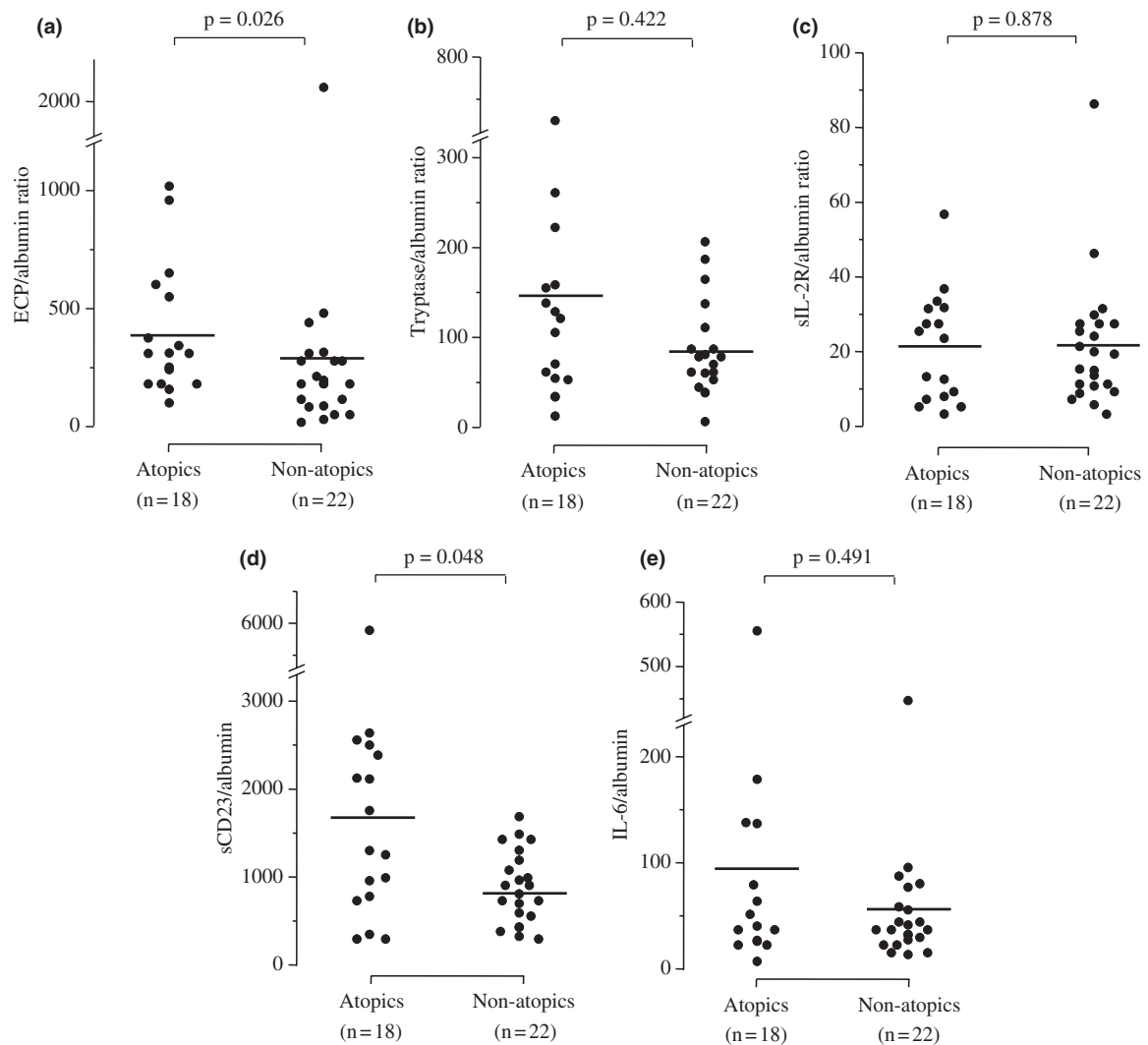


Fig. 3. The levels of inflammatory markers in adenoid tissue homogenates of atopic (n = 18) and non-atopic (n = 22) subjects. Markers include eosinophil cationic protein (ECP, a), mast cell-derived tryptase (b), T cell-associated soluble IL-2 receptors (sIL-2R, c), Th2 cell-associated soluble CD23 (sCD23, d), and B cell-associated IL-6 (e), which were expressed as the ratio to albumin. Group comparisons were performed by Mann-Whitney *U*-test. Horizontal bars indicate median values.

significantly higher in children with asthma sensitized to the house dust mite when compared with non-sensitized healthy children (16). Kitani et al. demonstrated that IgA antibodies to house dust mite in sputa were significantly higher in mite-sensitive patients than in normal controls and mite-insensitive asthmatic patients (17). In the present study, the levels of DP-specific IgE and IgA antibodies in adenoid tissue homogenate were significantly higher in atopics than in non-atopics, but not those of DP-specific IgG1 and IgG4, suggesting that these two antibodies have some role in immune responses in adenoid tissue. There have been few studies that demonstrate different cellular and cytokine profiles in the adenoids of allergic and non-allergic children (5, 6). Fokkens et al. saw a significant increase in

CD1a⁺ cells and eosinophils were more abundant in the adenoids of allergic children when tested using immunohistochemical staining methods. The other cell types investigated, such as CD4, CD8, CD-68, chymase, and tryptase did not show significant differences between the two groups (5). Similar cellular and cytokine profiles were noted within the middle ear effusions, the torus tubarius, and the adenoid tissues sampled with a predominant expression of IL-4 (6). In this study, the levels of ECP were significantly higher in atopics than in non-atopics, suggesting the role of activated eosinophil in adenoid inflammation of atopic children. No differences were found in inflammatory markers of the mast cell, T cell, and B cell. These findings suggest that the adenoids from atopic children may present

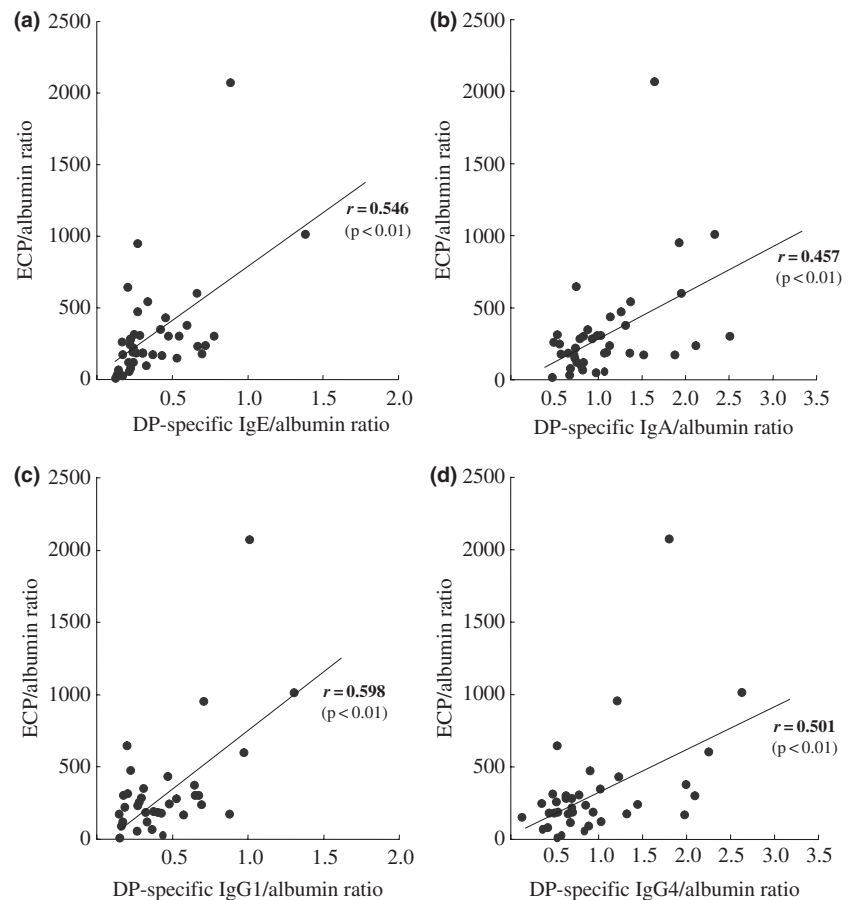


Fig. 4. Correlation between ECP, and DP-specific IgE (a), IgA (b), IgG1 (c), and IgG4 (d) levels in adenoid tissue from the study subjects.

different cellular and cytokine profiles than that of non-atopic children, as they present a more prominent eosinophilic inflammatory pattern.

It has been suggested that specific antibodies in surrounding tissue could stimulate eosinophil degranulation, resulting in the release of cytotoxic proteins such as ECP, eosinophil peroxidase, and eosinophil-derived neutrophils *in vitro* (18). IgA, particularly secretory IgA, has been shown to mediate eosinophil degranulation *in vitro* (19) and increased expression of IgA receptors on eosinophils of allergic individuals has been reported (20). Recent studies have implicated that IgG or IgA may be a contributor of allergic inflammation of the asthmatic airways or rhinitis (20–22). Bronchoalveolar lavage (BAL) fluids from asthmatic subjects allergic to ragweed could lead to a degranulation of eosinophils *in vitro*, and the degree of degranulation correlates with the levels of antigen-specific IgA, IgG, and IgG1 found in those fluids (23). Other BAL studies from allergic asthma patients have demonstrated that ragweed-specific IgE is most strongly correlated with ECP release and it has been reported that IgE receptors are present on eosinophils (24). In the present study, the levels

of DP-specific IgE, IgA, IgG1, and IgG4 correlate well with the levels of ECP in adenoid tissue homogenate, suggesting that specific IgE, IgA, and IgG found in adenoid tissue may be involved in eosinophilic inflammation of adenoid tissue.

In conclusion, we have confirmed the local production of total IgE and DP-specific antibodies, as well as the evidence of eosinophilic inflammation in adenoid tissue of atopic children. Moreover, locally-produced DP-specific IgE, IgA, and IgG antibodies may contribute to eosinophilic inflammation.

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